energy and 72.72 kJ/M energy was used in case of osteoarthritic samples. Loss of water content in all three groups are presented with a sharp step on the TG curve, starting on average temperature of 37 °C and ending at 116 °C. Linear part of the TG curve begun at around 62 °C and ended at around 112 °C. loss. In case of the normal hyaline cartilage 1.266%/1°C fluid loss was detected. In necrotic samples 1.689%/1 °C decrease in mass was observed. In the osteoarthritic 1.422%/1 °C mass reduction was measured

Conclusions: Increase in the cartilage matrix water content in all cases of degenerative articular cartilage was observed. Based on the results it can be stated that water content is higher in impaired samples, meanwhile water interstitial bonding was stronger in these cases. Activation energy correlated considerably with water content in the samples. The newly established thermogravimetric protocol was sufficient for compositional thermoanalytical study of normal and degenerative human hyaline cartilage. Previously, this method has not been used for this purpose. Characterization of the altered metabolism in cartilage that promote disease progression should lead to future treatment options that can prevent structural damage. Therapeutic steps can be adequately tested and monitored with thermogravimetric measurements.

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EVIDENCE FOR CLEAVAGE OF TYPE II COLLAGEN BY CATHEPSIN K IN HUMAN OSTEOARTHRITIC CARTILAGE

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Purpose: Cathepsin K is expressed in normal and osteoarthritic (OA) hyaline cartilage and is capable of cleaving type II collagen as well as other matrix molecules. The aim of this study was to determine whether there is evidence for cathepsin K- mediated cleavage of type II collagen in human OA cartilage.

Methods: Femoral condylar cartilages removed at arthroplasty for knee OA were cultured in serum-free medium in the presence and absence of a synthetic cathepsin K inhibitor (supplied by Merck Frosst, Montreal, Quebec, Canada). The content of a new type II collagen cleavage neoepitope that can be generated by cathepsin K was measured by ELISA assay. Aggrecan degradation was measured by the release of glycosaminoglycan using a colorimetric assay. Inhibitor toxicity was assessed by measuring the incorporation of [^{3}H] proline in cartilage cultured with and without the inhibitor. Type II collagen cleavage was also detected by ELISA and immunohistochemically in uncultured cartilages from both normal and OA knee joints.

Results: Cleavage of type II collagen was significantly enhanced in OA cartilage compared with healthy cartilage, as demonstrated by ELISA and immunolocalization. The inhibitor reduced collagen cleavage in cultures of 4 out of 8 patients, this being significant in 3 cases. There was no effect on proteoglycan release and the incorporation of tritiated proline was unaffected by the inhibitor.

Conclusions: These results show that cleavage of type II collagen at a site cleaved by cathepsin K is increased in OA articular cartilages. Based on the specificity and lack of detectable toxicity of the inhibitor, this cleavage is due in part to cathepsin K in almost half of the patients. Cathepsin K should therefore be considered as a potential therapeutic target in the control of cartilage degeneration in OA.

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BOVINE, PORCINE AND ICHTHYIC CHONDROITIN SULFATE DECREASE IL-1beta EFFECTS ON NO PRODUCTION AND APOPTOSIS: CORRELATION WITH MOLECULAR MODELING DATA

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Purpose: The current study examines whether porcine, bovine and ichthyic chondroitin sulfate (CS) would influence the production of nitric oxide (NO) and apoptosis in human osteoarthritic (OA) chondrocytes. Then, we confirm these results and explain them by a proposed novel activity concept of CS evaluated by molecular modeling.

Methods: Samples of human OA articular cartilage were obtained from patients undergoing knee arthroscopy. Firstly, OA human chondrocytes were incubated with porcine, bovine and ichthyic CS (100μg/mL) and stimulated with human recombinant Interleukin-1 beta (hrIL-1 beta) (10ng/mL), in the same time, to induce NO synthesis. NO release was measured as nitrite concentration in 24 and 48 hours culture supernatants by using the Griess reaction. Secondly, OA human chondrocytes were incubated with porcine, bovine and ichthyic CS during 72 hours and stimulated by various concentrations SNP (Sodium Nitroprusside) during 18 hours. SNP was used as a NO compound donor. To access the degree of apoptosis, APOPercentage Apoptosis Assay was used. This finding was further quantitatively confirmed by fluorescent microscopy using two apoptosis markers: TUNEL assay and Annexin-V fluos. In the third time, we modelized several CS oligosaccharides and we have tested their possibilities of interaction with IL-1 beta and its receptor.

Results: Bovine, porcine and ichthyic CS tested decreased significantly NO synthesis at 48 hours when human OA chondrocytes were cotreated with CS and hrIL-1beta. However, a preventive treatment with bovine, porcine and ichthyic CS during 72 hours and a stimulation with hrIL-1beta did not reduce significantly NO synthesis.

In OA chondrocytes treated with bovine, porcine and ichthyic CS, on average 18% of chondrocytes showed apoptotic features compared with 31% in chondrocytes treated with SNP. These data suggest that CS or CS oligosaccharides could interact with IL-1beta. The modeling study proposed two sites of interactions between IL-1 beta and some oligosaccharides. We have probed the specificity of this protein for distinct sulphatation sequences.

Conclusions: These results suggest that bovine, porcine and ichthyic CS prevent IL-1beta induced increase in NO production. This preliminary study suggests that bovine, porcine and ichthyic CS could downregulate apoptosis in the OA chondrocytes. A decrease of IL-1 beta effects could be the consequence of specific binding to oligosaccharides.

This study provides a plausible mechanism for the chondroprotective properties of bovine, porcine and ichthyic CS.

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CHONDROCYTE HYPERTROPHY - A NOVEL EX VIVO MODEL FOR EARLY CHANGES IN CHONDROCYTES IN OA

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Purpose: In early osteoarthritis (OA), hypertrophic chondrocytes are part of the pathology and are distributed throughout the

articular cartilage matrix. The de-differentiation of chondrocytes in OA may be an early event, that properly counteracted could provide a novel rationale and advance new drugs for OA. We wanted to develop a model system for early stage OA in an more *in-vivo* like *ex vivo* setting, using growth factors known to stimulate hypertrophy in various cell-lines.
Methods: Articular cartilage explants was harvested from bovine stifle joints and cultured with either bFGF [20 ng/mL], BMP-2 [20 ng/mL], or β-glycerol-phosphate [50 μg/mL] and ascorbic acid [10 mM] to stimulate the hypertrophic phenotype of the articular chondrocytes. Cultures was refreshed every other day and cultured for three weeks. The morphology was followed by histology by toluidine blue staining, and immunohistochemistry of hypertrophy markers: collagen type X, matrix metalloproteinase 13 (MMP-13), and alkaline phosphatase (ALP). Additionally, the CTX-II Ab was used to evaluate the contribution of collagen type II telopeptides fragments.
Results: Changes in morphology towards the hypertrophic phenotype after bFGF stimulation were associated with large chondrocytes and expression of collagen type X and ALP in the articular cartilage matrix. BMP-2 also stimulated hypertrophy in the articular cartilage explants, but only expression of ALP and MMP-13 was found. Hypertrophy induced by β-glycerol-phosphate and ascorbic acid, showed expression of all markers: ALP, MMP-13 Collagen type X (to a lesser extent), and CTX-II fragments. When all inducers of hypertrophy were combined (bFGF, BMP-2, β-glycerol-phosphate and ascorbic acid), changes in morphology into the hypertrophic phenotype were found, expression of ALP, MMP-13, and CTX-II, but not collagen type X.
Conclusions: Hypertrophic chondrocytes may be restricted to the articular cartilage in patients suffering from OA, and markers of hypertrophic chondrocytes may as such be sensitive and specific markers for early OA. The articular cartilage explants model can be used as a new system of early OA, that allow isolated investigations of potential novel treatments, which at best would reverse this pathological phenotype.

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REGULATION OF PROSTAGLANDIN E-2 PRODUCTION IN INTERLEUKIN ONE BETA ACTIVATED CHONDROCYTES PROPAGATED ON MICROCARRIER SPINNER CULTURE

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Purpose: The present study was designed to evaluate whether chondrocytes propagated in microcarrier spinner culture can be activated by IL-1β to produce PGE-2; and whether this activation can be blocked by natural products known to have anti-inflammatory activity: Avocado Soybean Unsaponifiables (ASU), glucosamine (Glu), and chondroitin sulfate (CS).
Methods: Canine chondrocytes ($4x10^3/cm^2$) seeded in collagen microcarrier beads were propagated in spinner culture for 14 days. They were next incubated with: media alone or the combination of ASU (NMX-1000™, 25μg/mL), CS (TRH122®, 20μg/mL) and Glu (FCHG49®, 10μg/mL). for 24 hrs. The combination of ASU, Glu, and CS was supplied by Nutramax Laboratories, Inc. Cultures were then incubated with media alone or activated with IL-1β (10 ng/mL) at 37°C, 5% CO_2 for 24 hrs. The supernatant was assayed for PGE-2 content. Chondrocytes were analyzed by microscopy and immunofluorescence for type II collagen. Data was analyzed by ANOVA with the Tukey post-hoc test. Values of $p<0.05$ were considered statistically significant.
Results: Chondrocytes attached, multiplied on microcarriers, and produced extracellular matrix material. The cultures formed aggregates and immunostained for type II collagen, indicating continued production of the protein. Activation of chondrocyte-seeded microcarriers at passage 3 and 4 showed similar responsiveness to the cytokine with IL-1β, PGE-2 levels of 179% and 165% of non-activated controls, respectively. Pretreatment of chondrocyte-seeded microcarriers with the combination of ASU, Glu, and CS significantly reduced PGE-2 levels to about 60% below non-activated controls ($p<0.05$).
Conclusions: The present study demonstrates that the microcarrier spinner culture system can be used to evaluate chondrocyte responses to pro-inflammatory stimuli and to identify agents that can modify these responses. The dynamic condition in the microcarrier spinner bioreactor appears to recapitulate the biomechanical environment that chondrocytes encounter in the joint. Therefore, the microcarrier spinner culture system may represent a useful tool to evaluate the potential anti-inflammatory properties of natural products. Using this culture system, we observed that the combination of ASU, Glu, and CS effectively blocks activation of the inflammatory pathway.

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INHIBITION OF MITOGEN KINASE PHOSPHATASE 1 (MKP-1) POTENCIATES CELL DEATH INDUCED BY TUMOR NECROSIS FACTOR α (TNFα) BUT NOT BY INTERLEUKIN 1 β (IL-1β) IN NORMAL HUMAN ARTICULAR CHONDROCYTES

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Purpose: Death of chondrocyte cells by apoptosis is a hallmark of degenerative joint diseases such as osteoarthritis (OA). Tumor necrosis factor α (TNF-α) and interleukin-1β (IL-1β) have been demonstrated to play a pivotal role in the development of OA disease. Previously we demonstrated that TNFα and IL-1β differently regulate Actinomicyn D (Act D)-mediated apoptosis of human chondrocytes.
Objective: This study addresses whether TNF-α and IL-1β differently modulate the cell death induced by Mitogen Kinase Phosphatase 1 inhibitor (Ro 31-8220) in normal human chondrocytes.
Methods: Normal human chondrocytes were isolated from knee cartilage obtained from necropsy from 16 adult cadavers (mean age 37 years). Ro (10 μg/ml) were used to induce apoptosis in chondrocytes. Apoptosis were evaluated by using flow cytometry (propidium iodide) and nuclear morphology was evaluated with 4',6'-Dianidino-2-phenylindole dihidrochloride (DAPI) by fluorescence microscopy. As a control, cell death was induced in Jurkat cells with staurosporine (1μM). Caspase-7, -3, bcl-2, mcl-1 and FLICE-inhibitory protein (FLIP) were analyzed by Western-blot.
Results: We demonstrated that the level of hypodiploid peak induced by TNF-α (10 ng/ml) + Ro increased significantly cell death induced by Ro (Ro: 7.99±5.3%; TNF-α+Ro: 23.05±7.3%, n=3, $p<0,0001$) at 24 hours. Notably, IL-1β (5 ng/ml) unlike TNF-α, not showed this synergistic effect with Ro (IL-1β+Ro: 5.93±3.93%). These results are in agree with nuclear morphological analysis, which demonstrated that the treatment with TNF-α+Ro resulted in a high number of cells condensed nuclei, not observed with IL-1β+Ro treatment. Furthermore, western blot studies indicated that IL-1β+Ro did not induce the activation of caspase-7 and -3 observed in a time dependent manner with TNF-α+Ro. On the other hand, this different effects were not due to the antiapoptotic proteins bcl-2, mcl-1 or FLIP apoptosis inhibitor protein that decrease in the same level in both conditions. To elucidate the role of the caspases on the effect of TNF-α on chondrocyte cell death induced by Ro, caspase inhibitors were employed (-3, -3/7, -8 and general) and hypodiploid DNA